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Serial Review: Alcohol, Oxidative Stress and Cell Injury
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DISTRIBUTION OF ETHANOL-INDUCED PROTEIN ADDUCTS IN VIVO: RELATIONSHIP TO TISSUE INJURY

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Abstract—Generation of oxygen free radicals and reactive aldehydes as a result of excessive ethanol consumption has been well established. Recent studies in human alcoholics and in experimental animal models have indicated that acetaldehyde, the first metabolite of ethanol, and the aldehydic products of lipid peroxidation can bind to proteins in tissues forming stable adducts. The demonstration of such adducts in zone 3 hepatocytes in alcoholics with an early phase of histological liver damage indicates that adduct formation may have an important role in the sequence of events leading to alcoholic liver disease. There may be interference with cellular functions, stimulation of fibrogenesis, and immunological responses. Autoantibodies towards distinct types of adducts have been shown to be associated with the severity of liver disease in alcoholic patients. High fat diet and/or iron supplementation combined with ethanol may increase the amount of aldehyde-derived epitopes and promote fibrogenesis in the liver. Recently, ethanol-derived protein modifications have also been found from other tissues exposed to ethanol and acetaldehyde, including rat brain after lifelong ethanol administration, pancreas, and rat muscle. Elevated adduct levels also occur in erythrocytes of alcoholics, which may be related to ethanol-induced morphological aberrations in hematopoiesis. © 2001 Elsevier Science Inc.

Keywords—Alcoholic liver disease, Free radical, Lipid peroxidation, Acetaldehyde, Malondialdehyde, Iron

INTRODUCTION

A growing body of evidence indicates that various distinct types of protein adducts are formed in vivo under conditions involving enhanced lipid peroxidation, glycation, or amino acid oxidation. This contribution will focus on current data on the generation of protein-aldehyde adducts in tissues as a result of excessive ethanol intake. The relationship between protein adducts and ethanol toxicity is also discussed.

DIFFERENT TYPES OF ETHANOL-INDUCED ADDUCTS IN ALCOHOLICS

Reactive compounds, which can bind to proteins, originate both from ethanol metabolism and ethanol-induced oxidative stress [1]. Upon binding with amino acid residues and nucleophilic biomolecules both stable and unstable condensates are generated [2]. To date, several distinct adduct types have been reported (Table 1). *Acetaldehyde (AA)*, the first metabolite of ethanol, binds to reactive lysine residues, to some aromatic amino acids, cysteine, or to free alpha-amino groups, such as the aminoterminal valine of hemoglobin [1–3]. Aldehydic products of lipid peroxidation, *malondialdehyde (MDA)* and *4-hydroxynonenal (HNE)*, also form Schiff's base adducts with proteins [1]. MDA originates from nonenzymatic lipid peroxidation of unsaturated fatty acids, from phagocytosis by monocytes and from arachidonic acid catabolism in platelets [4]. *Hybrid adducts*

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Table 1. Various Types of Protein Adducts Generated as a Result of Excessive Ethanol Consumption

Type of adduct	Abbreviation	Tissue distribution	Reference
Acetaldehyde	AA	Liver	
		Microsomal proteins	Zhu et al., 1996 [8], Jeong et al., 2000 [10]
		Hepatocytes with zone 3 predominance	Niemelä et al., 1991 [11], 1994 [12], 1995 [14], 1998 [15], Halsted et al., 1993 [13], Paradis et al., 1996 [16], Li et al., 1997 [19]
		Areas of fatty deposition and cytochrome enzyme induction	Niemelä et al., 1998 [15], 2000 [23]
		Areas of hepatitis in ALD, Ito cells	Paradis et al., 1996 [16]
		Sinusoids	Holstege et al., 1994 [18]
		Hepatocyte plasma membranes	Trudell et al., 1991 [17]
		Brain	Nakamura et al., 2000 [33], Rintala et al., 2000 [34]
		Gut	Biewald et al., 1998 [27]
		Muscle	Worrall et al., 2001 [35] Preedy et al. (this issue)
		Blood Cells and Molecules	Lin et al., 1990 [39], 1995 [41], Niemelä and Israel, 1992 [36], Latvala et al., 2001 [38]
Malondialdehyde	MDA	Liver	
		Hepatocytes with zone 3 predominance	Niemelä et al., 1994 [12], 1995 [14], 1998 [15], Tsukamoto et al., 1995 [24]
		Areas of hepatitis in ALD	Paradis et al., 1997 [20]
		Blood proteins	French et al., 1993 [40]
4-hydroxynonenal	HNE	Liver	
		Hepatocytes with zone 3 predominance	Niemelä et al., 1994 [12], 1995 [14], 1998 [15], Tsukamoto et al., 1995 [24], Paradis et al., 1997 [20], Li et al., 1997 [19]
		Ethanol-exposed fetal livers	Chen et al., 2000 [21]
		Pancreas, acinar cells	Casini et al., 2000 [30]
		Blood proteins	French et al., 1993 [40]
Malondialdehyde-acetaldehyde hybrid	MAA	Liver	
		Microsomes	Tuma et al., 1996 [5], Xu et al., 1998 [48]
		Atherosclerotic plaques	Hill et al., 1998 [42]
Hydroxyethyl radical	HER	Liver	
		Microsomes	Moncada et al., 1994 [6], Clot et al., 1995 [47], 1997 [7], Albano et al., 1996 [9]
		Hepatocyte plasma membranes	Clot et al., 1997 [7]
		Pancreas	Iimuro et al., 1996 [29]

with acetaldehyde and malondialdehyde have been designated as *MAA adducts* [5]. Oxidation of long-chain polyunsaturated fatty acids generates HNE, which reacts with protein sulfhydryl groups through a Michael addition mechanism [4]. In addition, *hydroxyethyl radicals (HER)*, which result from ethanol oxidation in the presence of iron, are capable of protein binding [6,7].

Although virtually all proteins can bind with reactive aldehydes in vitro, there seems to be some preferential targets in vivo [1]. These include erythrocyte membrane proteins, hemoglobin, albumin, tubulin, lipoproteins, collagens, ketosteroid reductase [1–3,8], and cytochrome enzymes, which also modulate the generation of the reactive compounds during ethanol metabolism [9,10].

TISSUE DISTRIBUTION OF ADDUCTS

Liver

Immunohistochemical studies have revealed AA-protein adducts in zone 3 hepatocytes in the early phase of alcoholic liver disease (ALD) [1,11–15]. Such adducts have been found in alcoholic patients with no obvious clinical or histological signs of liver disease [11]. In advanced ALD, adduct distribution is more irregular and widespread colocalizing with areas of hepatitis [11,16]. AA-modified epitopes may also occur on hepatocyte surfaces [17] and along the sinusoids being associated with the prognosis of the alcoholic patients [18]. In rats, a 4 week administration of an ethanol-containing diet has been shown to lead to the formation of both centrilobular and sinusoidal AA adducts, which tended to be most abundant in those showing withdrawal symptoms during the experiment, suggesting a link between blood alcohol levels and the generation of hepatic protein adducts [12].

Oxidative modification of proteins with MDA and 4-HNE were first demonstrated from arterial vessel walls of atherosclerotic lesions (see [4]), and later from hepatic tissue in ALD [12,14,15,19,20], ethanol-exposed fetal livers [21], and iron overload [22]. Double immunofluorescence stainings for AA and MDA adducts in ALD

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have revealed a significant colocalization between these adducts at sites of histological changes [12]. MAA adducts are also generated in vivo [5]. HER adducts have been found from liver microsomes and hepatocyte plasma membranes of ethanol-fed animals [6,7].

High-fat diet together with ethanol stimulates several types of adducts together with increased expressions of cytochrome P450IIE1 and 3A [15,19]. In humans, protein adduct formation is also associated with induction of cytochromes 2E1, 2A, and 3A [23]. When a high-fat diet is further combined with iron, adduct formation is strikingly potenciated coinciding with elevated levels of serum liver-derived enzymes and progressive histopathology, including micronodular cirrhosis in rats [24]. Ethanol and iron may have a synergistic effect in producing oxidative stress, proinflammatory cytokines, and liver pathology. Tissue deposition of iron alone in experimental [25] or hereditary [22] hemochromatosis leads to the appearance of only rather small amounts of lipid-peroxidation derived aldehydes. Interestingly, *non-alcoholic steato-hepatitis* (NASH), which is typically found in obese or diabetic patients, resembles ALD with respect to the production of protein adducts, oxidative stress, and cytochrome enzyme induction in the same vicinity [23].

A follow-up study in a micropig model of ALD developed by Halsted and coworkers revealed progressive accumulation of adducts with AA, MDA, and 4-HNE upon prolonged ethanol intake [14]. After 1 month, AA and MDA adducts were found in zone 3 hepatocytes coinciding with increased serum AST and ALT. The adducts also co-localized with the sites of subsequent collagen deposition [13,14]. Perivenous fibrosis was noted especially in the animals, which had developed adducts of AA and MDA in the early phases of follow-up, suggesting a link between adduct formation and fibrogenesis. The most abundant amounts of adducts occurred in castrated minipigs also showing steatonecrosis, inflammation, and cytochrome enzyme induction, indicating that sex steroid hormones also influence adduct formation and liver toxicity [26].

Alimentary tract and pancreas

So far, only limited information is available on the occurrence of protein adducts in the alimentary tract [27] and pancreas [28–30]. Intestinal bacteria may be able to metabolize ethanol, thereby increasing AA concentrations and local toxicity in the gut [31]. Interestingly, extrahepatic AA, as administered in drinking water, has also been shown to result in hepatotoxicity and protein adduct formation [32]. In pancreas, ethanol intake leads to the formation of HER [29] and HNE [30] adducts, suggesting a role for free radical generation in ethanol-induced pancreatitis. Lipid peroxidation-derived adducts also occur in pancreatic iron overload [28].

Brain

Evidence of AA-protein adduct formation in rat brain has been recently provided by two groups of investigators [33,34]. After lifelong ethanol consumption such reactions were found in the white matter, large neurons in the deep layers of the frontal cortex, and in the molecular layer of the cerebellum, particularly in a rat strain (ANA), which usually shows high concentrations of AA during ethanol oxidation [34].

Muscle

Ethanol metabolites and enhanced lipid peroxidation may also be involved in the generation of myopathy in alcoholics, as evidenced by the demonstration of adducts in experimental ethanol-induced myopathy [35]. These observations are discussed in detail elsewhere in this forum by Preedy et al.

Blood cells and molecules

Protein-AA adducts have been found in erythrocytes after heavy drinking bouts [36]. Such adducts also occurred in alcoholic women who subsequently gave birth to children with fetal alcohol effects [37]. In immunocytochemical analyses of peripheral blood erythrocytes and bone marrow cells, AA-derived epitopes were found both inside the red cells and on cell membranes, which may be associated with the morphological erythrocyte abnormalities in alcoholics [38].

Adduction of serum proteins is expected to occur particularly in proteins which are synthesized in the liver, including albumin, transferrin, and lipoproteins [1,3,39, 40]. Lipoproteins may contain several types of modifications, which could create immunological responses, activate apolipoprotein E synthesis in macrophages, and induce inflammatory responses, thereby promoting atherogenesis in alcohol abusers [41,42]. In fact, recent studies on atherosclerosis have provided strong evidence pointing to a pathogenic role of "oxidized LDL," which represents a mixture of aldehyde-LDL adduction.

IMPLICATIONS OF ADDUCT FORMATION IN TISSUES

Disturbed cellular functions

As a consequence of adduct formation, the physicochemical properties of proteins, nucleic acids, and lipids may be altered [2]. Protein function is disturbed particularly when a lysine residue is in a functionally critical

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location, such as in tubulin and in lysine-dependent enzymes. Adduction of microtubules may impair protein secretion and plasma membrane assembly. Aldehyde-DNA binding has been suggested to promote carcinogenesis in alcoholics [43]. Several lines of recent evidence have indicated that Kupffer cells are highly responsive to the effects of ethanol and play a major role in the pathogenesis of ALD, including the release of cytokines, which may be stimulated by reactive oxygen species. Although as yet the links between adduct formation and Kupffer cell responses have remained unknown it is notable that Kupffer cell inactivation by gadolinium chloride leads to a decrease in hepatic protein adducts (O. Niemelä, S. Parkkila, B. Bradford, R.G. Thurman, unpublished results).

Fibrogenesis

Alcoholic patients develop varying amounts of liver fibrosis even upon equal amounts of ethanol consumption. On the other hand, early fibrosis in perivenous hepatocytes may predict subsequent development to cirrhosis [44]. Therefore, it is of interest to note that this zone is also the first site of protein adduct deposition. Hepatic stellate cells (Ito cells), which are the primary source of the extracellular matrix, are activated by AA and oxidative stress (for references, see [1]). In cell culture, AA and the lipid peroxidation products increase collagen mRNA levels and the expression of connective tissue proteins, whereas inhibition of adduct formation by scavengers of reducing equivalents prevents such increases.

Immune responses

Both humoral and cell-mediated immunological responses against the various types of protein adducts are stimulated by ethanol intake [3,7,45–51]. Circulating immunoglobulins with anti-AA- [3], or anti-MAA-adduct [48] specificity have been found in ethanol-fed rats. Autoantibodies recognizing AA- [45,49,50], MDA- [50], MAA- [51], or cytochrome P450IIE1-HER adducts [47] are generated in human alcoholics. The immune responses against AA adducts consist primarily of IgA and to some extent also from IgG antibodies [46,47,49], whereas the MDA-epitopes may create more IgG isotype responses [50]. Anti-AA-adduct IgA titers have been shown to be elevated in 69% of ALD patients [49], the titers being significantly higher than those in patients with nonalcoholic liver disease, heavy drinkers without liver disease, or nondrinking controls. The antibody titers also correlate with the severity of ALD, as measured with clinical and laboratory indices [49], or with the degree inflammation and necrosis in biopsy specimens [49–51].

Diagnostic implications

The demonstration of specific protein adducts in alcoholics has stimulated research on using such condensates as biological markers of ethanol-induced diseases. However, so far adduct assays have shown insufficient sensitivities for such purposes. Autoimmune responses, most specifically the IgA responses to AA adducts, may prove useful to differentiate between alcoholic and nonalcoholic liver diseases [49]. Only few studies are, however, available to compare adduct compositions and tissue distributions in different types of liver diseases. At least MDA and HNE adducts are found in several conditions, including viral liver disease, primary biliary cirrhosis, hemochromatosis, and NASH [20,22,23].

CONCLUSIONS

Ethanol abuse leads to the production of several types of protein adducts in tissues and in circulating proteins, which may contribute to the adverse effects of ethanol in tissues. Further studies on the rates of adduct formation and on their relative importance in vivo may result in potential clinical applications for more specific detection of ethanol-induced diseases. It also remains to be established whether modulation of adduct formation could aid in therapeutic interventions in alcoholic patients.

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ABBREVIATIONS

AA—acetaldehyde
ALD—alcoholic liver disease
ALT—alanine aminotransferase
AST—aspartate aminotransferase
4-HNE—4-hydroxynonenal
LDL—low-density lipoprotein
MAA—hybrid adduct with malondialdehyde and acetaldehyde
MDA—malondialdehyde
NASH—non-alcoholic steatohepatitis

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Source: https://www.industrydocuments.ucsf.edu/docs/fsnx0001